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# Effects of $\alpha$ -amino acids and small peptides on the rate of an $S_N1$ acetal hydrolysis reaction in aqueous solution: the interplay of hydrophobic and hydrophilic solute hydration

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The effects of small amounts of anionic  $\alpha$ -amino acids and several small peptides on the kinetics of the  $S_N1$  hydrolysis of 2-(4-nitrophenoxy)tetrahydropyran have been investigated at pH 11 and 40 °C. The rate-retarding effect at 1 molal of cosolute is plotted as  $\ln(k_{m=1}/k_{m=0})$  versus the number of CH groups in the amino acid side chain. Linear correlations are observed for small  $\alpha$ -amino acids from Gly up to Pro. Additivity is also obtained for longer alkyl chains with  $n(\text{CH}) > 6$ , but these retarding effects of the CH groups are larger and comparable to the CH group contribution obtained for short-chain primary alcohols. The kinetic effects of isomeric aliphatic  $\alpha$ -amino acids with linear and branched side chains are compared and show non-additivity. The results are interpreted in terms of the hydrophobicity of CH groups inside and outside the hydrophilic hydration spheres of the polar groups of the  $\alpha$ -amino acid. Amino acids with aromatic side chains do not fit in the additivity pattern, probably due to their more pronounced hydrophobicity.

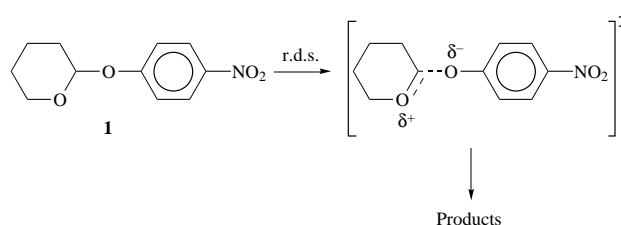
Kinetic data for two isomeric dipeptides, Gly-Val and Val-Gly, are also rationalised in terms of intramolecular hydration shell overlap and show the interplay of hydrophobic and electrostatic interactions. The effects of glycine oligomers,  $(\text{Gly})_n$  ( $n = 2, 3, 4$ ), reveal the complexity of the hydration of multi-functional molecules. The results are relevant in the context of understanding molecular recognition processes involving enzymes and proteins in aqueous solution.

## Introduction

Studies of the aqueous solvation of  $\alpha$ -amino acids<sup>1</sup> are very important for the understanding of processes such as protein folding and enzyme-substrate recognition. Solvent-induced hydrophobic interactions between apolar amino acid side chains provide probably the dominant force in protein folding,<sup>2-5</sup> though the molecular mechanism of hydrophobic interactions is still a matter for debate.<sup>6</sup> The opinion that intramolecular hydrophilic interactions (by groups that can form hydrogen bonds) are probably more significant in biochemical processes than hydrophobic interactions is, however, still held.<sup>7</sup> Numerous hydrophobicity scales have been developed for the primary building units of proteins, the  $\alpha$ -amino acids. These are based on physical properties including transfer parameters,<sup>8</sup> partition coefficients<sup>9,10</sup> and surface activities of amino acid-derived surfactants.<sup>11</sup> An overview of hydrophobicity scales for  $\alpha$ -amino acids has been given by several authors.<sup>12-14</sup>

These scales are usually dependent on the method. Therefore there is a need for more information on the hydration properties of  $\alpha$ -amino acids and particularly for the (in)dependent hydration properties of their constituent functional groups.

In this investigation we obtained information on  $\alpha$ -amino acid hydration by studying the interactions of  $\alpha$ -amino acids with other solutes in aqueous solution by measuring kinetic medium effects. Previously we investigated the kinetics of the hydrolysis of an activated amide in the presence of  $\alpha$ -amino acids at pH 4.<sup>15,16</sup> It turned out that non-covalent interactions of the side chain with the reactant are often overwhelmed by electrostatic interactions involving the carboxylate and ammonium moieties. The zwitterionic character of  $\alpha$ -amino acids in water obscures side-chain interactions, apparently due to the extensive hydration shells of the charged groups. In the present study we investigated the unimolecular hydrolysis of 2-(4-nitrophenoxy)tetrahydropyran (Scheme 1) in the presence of



**Scheme 1** Reaction mechanism of the unimolecular hydrolysis of **1**

small amounts of simple alcohols,  $\alpha$ -amino acids and small peptides at pH 11 and 40 °C. At low pH ( $\text{pH} < 4$ ), the reaction is subject to general acid catalysis;<sup>17</sup> at higher pH the reaction is pH-independent<sup>18,19</sup> and at pH 11 the formation of the 4-nitrophenoxide anion can be conveniently monitored by visible spectroscopy. The rate-determining step is the unimolecular breakage of the C–O bond *via* a dipolar and late transition state.<sup>18</sup> Bond breakage is far advanced, if not complete in the transition state. Previously we developed a theory in which kinetic data can be quantitatively described by a Gibbs energy interaction parameter<sup>20,21</sup>  $G(C)$ , which is the difference in pairwise interaction Gibbs energy between the cosolute and the initial state and the cosolute and the transition state of the reaction [eqn. (1)]. In this equation  $m$  is the molality of

$$\ln(k/k_{m=0}) = 2G(C)m/RT - nM_w\phi m \quad (1)$$

cosolute,  $n$  is the number of water molecules involved in the transition state of the hydrolysis reaction,  $\phi$  is the osmotic coefficient, which equals 1 in dilute solution, and  $M_w$  the molecular mass of water (the second term reflects the effect of the cosolute on the reactivity of water). If the quantitative description of pairwise interactions in terms of a Gibbs energy interaction

parameter can be applied to this hydrolysis reaction as it was for the hydrolysis of substituted acyl triazoles,<sup>22</sup> a linear dependence of  $\ln(k_m/k_{m=0})$  on the molality of the additive should be observed. As water is not involved in the rate-determining step, the term for the effect of the addition of a cosolute on the reactivity of water can be omitted and the relation simplifies to eqn. (2). First, we examined the effect of short-chain primary

$$\ln(k/k_{m=0}) = 2G(C)m/RT \quad (2)$$

alcohols on the hydrolysis of **1** in order to check the applicability of the quantitative description in terms of the solute-solute pairwise interactions on this hydrolysis reaction. In all cases the alcohols decreased the rate of the hydrolysis. Linear correlations between  $\ln(k/k_{m=0})$  and the molality of cosolute were observed.  $G(C)$  values were obtained from the slopes and following the group additivity approach as formulated by Savage and Wood,<sup>23</sup> satisfactory additivity was obtained for the group contribution by the CH moiety to the rate-retarding effect. Results for the  $\alpha$ -amino acids and peptides were much more complex and showed a subtle interplay between hydrophobic and hydrophilic hydration properties of these solutes.

## Experimental

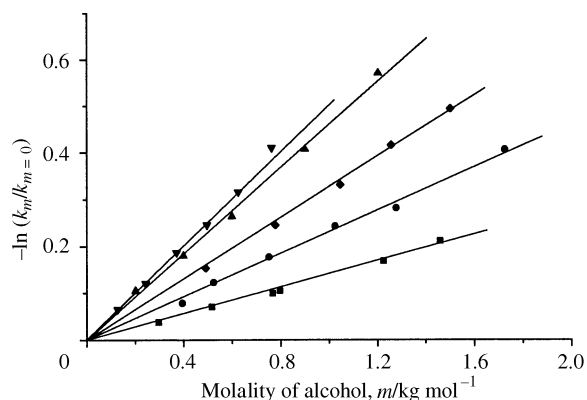
All amino acids and peptides were purchased from Janssen Chimica, Fluka and Sigma and were used without further purification (purity of 99% or higher). 2-(4-Nitrophenoxy)tetrahydropyran (**1**) was synthesised according to literature procedures.<sup>17</sup> Solutions for the kinetic measurements were made up by weight using deionised water, adjusted to pH 11 with a NaOH solution and were prepared immediately prior to use. About 5–8  $\mu$ l of a stock solution containing **1** in acetonitrile was injected into 2.5 ml of reaction medium and placed in a thermostatted cell compartment (40.0 °C) of a Perkin-Elmer  $\lambda$ 5 or  $\lambda$ 2 spectrophotometer. All kinetic measurements were performed while monitoring the reaction in the absence of a cosolute in the same kinetic run. Excellent first-order kinetics were obtained by following the change in absorbance at 400 nm. Rate constants were calculated using a data station connected to the  $\lambda$ 5 and a fitting program on the  $\lambda$ 2 spectrophotometer and were reproducible to within 1% for solutions containing the  $\alpha$ -amino acids and 2% for the peptides and alcohols.

## Results and discussion

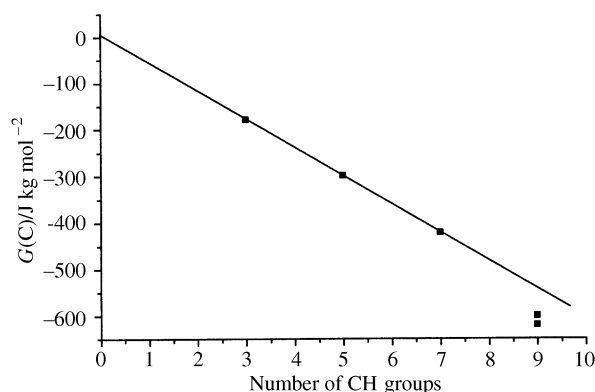
For the hydrolysis of **1** in mixtures of water with MeOH, EtOH, 1-PrOH, 1-BuOH and 2-BuOH, rate constants were determined at several molalities up to *ca.* 1.5 molal of cosolute, except for 1-BuOH which was measured only up to 0.75 molal due to solubility constraints. All alcohols retarded the rate of hydrolysis as was anticipated on the basis of their hydrophobic nature; hydrophobic interactions with the apolar initial state of the acetal hydrolysis will be more pronounced than with the dipolar transition state. Plots of  $\ln(k_m/k_{m=0})$  versus the molality of alcohol gave excellent linear correlations (Fig. 1).  $G(C)$  values were obtained from the slopes of these plots (Table 1). A plot of these  $G(C)$  values versus the number of CH groups in the cosolute confirms the additivity of the Gibbs energy contribution of the CH groups to the medium effect (Fig. 2). Accordingly, pairwise group interaction parameters were obtained *i.e.*  $G(\text{CH})$  and  $G(\text{OH})$ , which are  $-61 \pm 9$  and  $4 \pm 5$  J kg mol<sup>-2</sup>, respectively. In other words, the CH group has a negative contribution to the observed rate effect, which can be explained by dominating stabilising hydrophobic interactions between the cosolute and the initial state. Apparently, the OH group of the alcohols has a similar interaction with the initial state and the transition state because its contribution to the overall solvent effect is negligible. This contrasts with the effects of alcohols on the hydrolysis of 1-benzoyl-3-phenyl-1,2,4-

**Table 1** Pairwise Gibbs energy interaction parameters for primary alcohols and the relative retardations at 1 molal concentration

Alcohol	$G(C)/\text{J kg mol}^{-2}$	$\ln(k_{m=1}/k_{m=0})$
MeOH	$-179 \pm 8$	-0.14
EtOH	$-299 \pm 11$	-0.24
1-PrOH	$-422 \pm 14$	-0.34
1-BuOH	$-621 \pm 7$	-0.50
2-BuOH	$-601 \pm 20$	-0.49



**Fig. 1** Effects of methanol (■), ethanol (●), propan-1-ol (◆), butan-2-ol (▲) and butan-1-ol (▼) on the hydrolysis of **1**



**Fig. 2** Pairwise Gibbs energy interaction parameters as a function of the number of CH groups in the alcohol

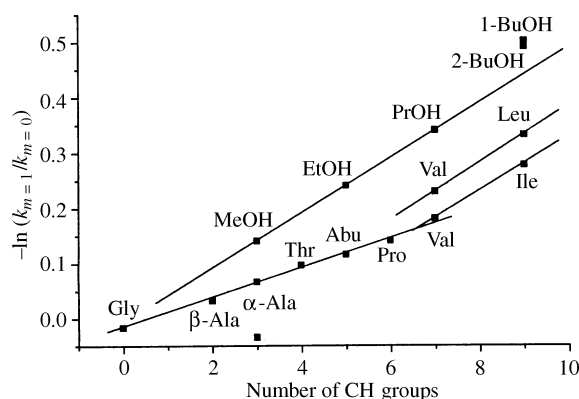
triazole.<sup>22</sup> Although for these activated amides approximately the same  $G(\text{CH})$  was obtained ( $-68$  J kg mol<sup>-2</sup>), a much larger positive value for  $G(\text{OH})$ ,  $226$  J kg mol<sup>-2</sup>, was calculated. This difference may be rationalised in terms of the reaction mechanism of the amide hydrolysis, which involves two water molecules in the transition state.<sup>24</sup> The Gibbs energies of interaction of the alcoholic OH group with the initial state and the transition state of the amide hydrolysis reaction will therefore differ from those of the acetal hydrolysis as there are favourable interactions between the cosolute OH group and the substrate transition state OH groups.

Secondly, we measured the effect of a series of  $\alpha$ -amino acids on the rate of acetal hydrolysis. At pH 11 the  $\alpha$ -amino acids are not zwitterionic but anionic solutes and solute-solvent and solute-solute interactions are expected to differ significantly from those of the zwitterionic form. The effects of the  $\alpha$ -amino acids on the kinetics of the hydrolysis of **1** do not exhibit linear correlations between the  $\ln(k_m/k_{m=0})$  and the molality of the  $\alpha$ -amino acid, but instead show a curvature which increases with increasing molality and hydrophobicity of the  $\alpha$ -amino acid. Therefore, the quantitative description of the pairwise interactions used to analyse the results for the alcohols cannot be applied to the amino acids and consequently no  $G(C)$  values could be obtained. In order to quantify the relative rate retar-

**Table 2**  $\ln(k_{m=1}/k_{m=0})$  of **1** in water containing 1 molal amino acid (or dipeptide)

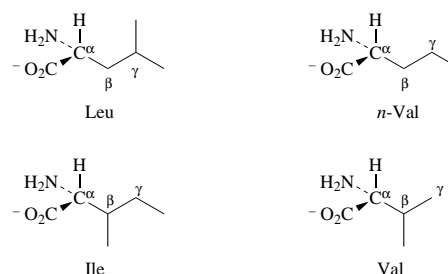
Cosolute	$\ln(k_{m=1}/k_{m=0})$
Gly	+0.010
$\beta$ -Ala	-0.032
$\alpha$ -Ala	-0.066
Thr	-0.096
Abu <sup>a</sup>	-0.115
Pro	-0.140
Aiba <sup>b</sup>	-0.098
Val	-0.180
<i>n</i> -Val	-0.229
Ile	-0.277
Leu	-0.331
Leu <sup>c</sup>	-0.142
<i>n</i> -Leu <sup>c</sup>	-0.164
Phe <sup>c</sup>	-0.315
$\beta$ -Phenylserine <sup>c</sup>	-0.230
Lys	-0.174
Gly-Val	-0.300
Val-Gly	-0.361
Gly-Gly	+0.102
Gly-Gly-Gly	-0.188
Gly-Gly-Gly-Gly	-0.185
<i>N</i> -Methyl Gly	-0.069
<i>N,N</i> -Dimethyl Gly	-0.139
<i>N,N,N</i> -Trimethyl Gly	-0.055
NaAc	+0.035

<sup>a</sup> Abu =  $\alpha$ -Aminobutyric acid. <sup>b</sup> Aiba =  $\alpha$ -Aminoisobutyric acid. <sup>c</sup> Values at 0.5 molal of added amino acid.

**Fig. 3** Relationship between the number of CH groups in the  $\alpha$ -amino acid side chain and its effect on the rate of hydrolysis of **1** at 40 °C and pH 11

dations induced by the additives, the natural logarithms of the relative rates at 1 molal of  $\alpha$ -amino acid were plotted against the number of CH groups in the  $\alpha$ -amino acid side chain. At this concentration the rate differences between the different cosolutes are quite pronounced. The number of CH groups in Gly was assigned 0, since it has no side chain,  $\alpha$ -Ala has 3 CH groups *etc.* The number of CH groups in  $\beta$ -Ala was arbitrarily set as 2. The results are displayed in Table 2 and Fig. 3. For comparison, the corresponding values for the alcohols have been included in this figure. The anionic  $\alpha$ -amino acids have a retarding effect on the reaction. Presumably hydrophobic interactions between the  $\alpha$ -amino acid side chain and the initial state dominate the medium effect. Gly has a negligible effect on the reaction rate and can be considered as non-hydrophobic, if not hydrophilic. Clearly, the effect caused by the  $\alpha$ -amino acids is smaller than that caused by alcohols with the same number of CH groups. We contend that the free amino group and the carboxylate group are extensively hydrated at pH 11 and camouflage the interactions of the apolar groups with the reactant. Additivity within the amino acid series is observed with a break at  $n(\text{CH}) = 6$ . It is remarkable to see that proline (imino acid) fits in the range though it is on the breakpoint of the two lines.

The fact that Thr fits in is anticipated as we noted already that the contribution of the OH group to the kinetic solvent effect is negligible. In terms of the additivity rules isomers should exhibit similar rate effects, but we find that when isomers of the  $\alpha$ -amino acids with longer alkyl chains are considered, they exert different effects on the rate of hydrolysis. This observation, as well as the breakpoint in the linear correlation (Fig. 3) can be rationalised as follows. The additivity of the CH-contribution to the solvent effect for  $n(\text{CH}) \leq 6$  is a 'masked' contribution. These CH groups are all situated within the hydrophilic hydration spheres of the  $-\text{NH}_2$  and  $-\text{CO}_2^-$  groups. The hydrophobic hydration shells of the CH moieties are therefore badly developed and their retarding effect on the hydrolysis is diminished. Consequently, they possess a lower apparent hydrophobicity. When  $n(\text{CH}) > 6$ , the additional CH groups are presumably situated outside the hydration spheres of the polar groups and now their hydrophobicity equals the hydrophobicity of the CH group in the alcohols.<sup>25</sup> We observed previously that the effect of ionic group hydration on the hydrophobic interactions of nearby methylene groups leads to non-additivity.<sup>26-28</sup> The importance of ionic hydration in  $\alpha$ -amino acids on the apparent hydrophobicity of nearby apolar groups has also been observed by other investigators.<sup>29-31</sup> The results for the isomers (Val/*n*-Val† and Ile/Leu) support this theory. Whereas the side chains of Val and Ile are branched on the  $\beta$ -carbon atom, Leu is branched on the  $\gamma$ -atom and *n*-Val has a chain elongation on  $\gamma$ -C. Introduction of a methyl group on the  $\beta$ -C atom represents

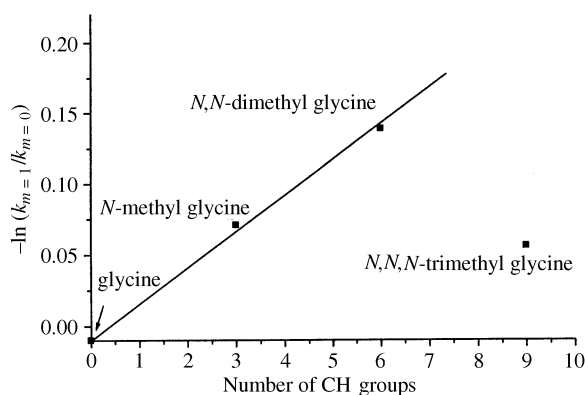


an extension of the alkyl chain within the hydration spheres of the polar groups, whereas introduction of a methyl group on  $\gamma$ -C is an extension of the alkyl chain outside the influence spheres of the polar groups. In Fig. 3 one could also connect the data points obtained for *n*-Val and Ile which represents the introduction of a CH group within the hydration layers of the polar groups; this line should run parallel to the line connecting the data for the  $\alpha$ -amino acids with  $n(\text{CH}) \leq 6$ . This is indeed borne out in practice and is consistent with the ideas postulated above. Because the solubility of *n*-Leu† at pH 11 is insufficient to measure its kinetic effect at 1 molal, both *n*-Leu and Leu were measured at 0.5 molal. It was anticipated that these isomers have similar effects on the reaction rate. In fact *n*-Leu retards the reaction slightly more efficiently than Leu (Table 2). Probably the polar hydration spheres even extend to larger distances, but the availability and solubility of longer-chain  $\alpha$ -amino acids are limited and prevent more detailed investigation.

The retardation caused by Aiba ( $\alpha$ -aminoisobutyric acid), an isomer of Abu ( $\alpha$ -aminobutyric acid) and the only amino acid which has two substituents on  $\alpha$ -C, is slightly less than anticipated on the basis of additivity. We observed previously that this  $\alpha$ -amino acid shows a deviant behaviour from that of the common  $\alpha$ -amino acids.<sup>16</sup>

Sodium acetate slightly accelerates the reaction. This indicates that the amino group in the  $\alpha$ -amino acids must exert a slightly decelerating effect. However, this conclusion is un-

† IUPAC names: *n*-Val (norvaline) = 2-aminopentanoic acid; *n*-Leu (norleucine) = 2-aminohexanoic acid.



**Fig. 4** Relationship between the number of CH groups in *N*-methylated glycines and their effect on the rate of hydrolysis of **1** at 40 °C and pH 11

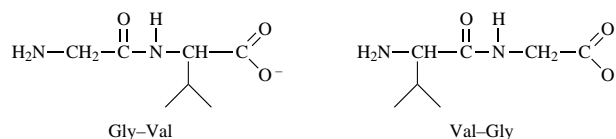
certain as we have no information about the mutual interactions of the incompatible hydration spheres of  $-\text{NH}_2$  and  $-\text{CO}_2^-$ , which at least partly overlap. From the absence of a significant rate effect caused by Gly we conclude that the combined effects of the two polar groups in the amino acid molecule cancel (the central  $\text{CH}_2$  group is apparently not available for any type of interaction with the substrate). On the other hand, extrapolation from  $\text{Leu} \rightarrow n\text{-Val} \rightarrow \text{Abu}$  to  $n(\text{CH}) = 0$ , which is more fair because it deals with the additivity of methylene groups that are not influenced by the ionic hydration, we obtain a contribution of  $(-\text{NH}_2 + -\text{CO}_2^-)$  which is clearly positive. This pattern can be explained by favourable electrostatic interactions between the carboxylate and amino group of the amino acid with the transition state of the reaction. This argument implies that not only hydrophilic hydration reduces hydrophobic interactions of nearby methylene groups, but that the effect is mutual: hydrophobic groups can influence hydrophilic hydration as well.

We have also measured the kinetic effects exerted by Phe and  $\beta$ -phenylserine. These cosolutes have low solubilities in water and so their kinetic medium effects were measured at 0.5 molal (Table 2). Even at these low molalities it is obvious that these cosolutes cannot be incorporated into the additivity series for the aliphatic  $\alpha$ -amino acid chains. They retard the hydrolysis to a significantly larger extent than anticipated on the basis of this additivity scheme. The distinctive behaviour of these  $\alpha$ -amino acids has also been observed for the hydrolysis of 1-benzoyl-3-phenyl-1,2,4-triazole and has been attributed to the pronounced hydrophobicity of these cosolutes.<sup>15</sup>

Interesting results were obtained with the *N*-methylated glycines as cosolutes. *N*-Methyl glycine, *N,N*-dimethyl glycine and *N,N,N*-trimethyl glycine were added in a 1 molal concentration to the reaction medium. In Fig. 4 the values of  $\ln(k_{m=1}/k_{m=0})$  are plotted against the number of CH groups in the nitrogen substituents. Up to  $n(\text{CH}) = 6$  additivity of the CH group contribution is observed and the values perfectly overlap with the values displayed in Fig. 3 for Ala and Pro. This seems to indicate that the position of the methyl group, that is to say whether it is positioned on the  $\alpha$ -carbon atom or on the nitrogen atom, does not influence its apparent hydrophobicity. However, *N,N,N*-trimethyl glycine shows a significant deviation from the observed additivity in this series. Two major structural features distinguish this cosolute from the less substituted glycines. First, the hydrogen bond acceptor ability of the amine group has vanished, because the non-bonded free electron pair is used to accommodate the third methyl group. Secondly, the solute is now a zwitterionic species. The latter is most probably responsible for the observed decrease in rate retardation. The ammonium group will be hydrated differently and to such an extent that the hydrophobic hydration of the methyl groups is reduced

in comparison to *N*-methyl glycine and *N,N*-dimethyl glycine. These results indicate that ionic (polar) hydration has a more destructive influence on hydrophobic hydration than has non-ionic polar hydration.

Finally we have determined kinetic data for two dipeptides Gly-Val and Val-Gly and three glycine oligomers; diglycine,



triglycine and tetraglycine (Table 2). As Gly does not exert a measurable effect on the hydrolysis reaction, it was anticipated that the two dipeptide isomers would cause a retardation quite similar to that caused by Val. However, both isomers retard the reaction to a significantly larger extent than Val. It is difficult to ascribe this increase in retardation to a particular structural feature in the dipeptide. An additional amide functionality has been introduced in the molecule and the mutual interactions of the functional groups with each other have changed as well. However, the difference between the two isomeric dipeptides can be satisfactorily explained. In Gly-Val the isopropyl group experiences more shielding by the carboxylate group than in Val-Gly, where the isopropyl group is more remote from the extensively hydrated negative charge and hence its higher apparent hydrophobicity is reflected in a stronger rate retardation.

The different contributions of the methyl substituents in Gly-Ala and Ala-Gly to the overall hydrophobicity of the cosolute have been observed previously by others and was explained in terms of the influence of the different hydration properties of the polar groups of the dipeptide on the methyl hydrophobicity as well.<sup>30</sup> Once more, these results support the idea that intramolecular hydration shell overlap markedly influences the non-covalent interactions of the  $\alpha$ -amino acid side chain with the substrate.

The effects of the glycine oligomers exhibit a complex interaction pattern. Diglycine significantly accelerates the reaction whereas tri- and tetra-glycine show a large and similar retardation of the hydrolysis reaction (Table 2). Intramolecular hydrogen bonding interactions in short peptides in aqueous solution are highly unlikely, particularly in the absence of residues with an apolar side chain as is the case in the glycine oligomers. Since intermolecular interactions may play a role at 1 molal of the higher glycine oligomers, one has to consider that in triglycine and tetraglycine the number of amide functionalities is two and three times as large as in diglycine, respectively. A small peptide aggregate might create a favourable environment for the substrate where the initial state is more stabilised than the transition state. However, this still leaves the acceleration observed for diglycine unexplained.

It is clear that more data on peptides as cosolutes are required in addition to the present preliminary data to obtain a more complete picture of the dominant non-covalent interactions of peptides in aqueous solutions and how these interactions affect the hydrolytic process.

## Conclusions

In this study we have investigated the effect of primary alcohols and a series of  $\alpha$ -amino acids on the kinetics of hydrolysis of 2-(4-nitrophenoxy)tetrahydropyran, a unimolecular  $\text{S}_{\text{N}}1$  hydrolytic process. For the alcohols, the rate retardations were analysed in terms of pairwise Gibbs energy interaction parameters, reflecting the difference in interaction of the cosolute with the initial state and the transition state of the reaction. With the additivity theory formulated by Savage and Wood,<sup>23</sup> pairwise group interaction parameters were obtained. The contribution

of the hydroxy group to the solvent effect is negligible, whereas the contribution of the CH group is significant, namely  $-61 \pm 9 \text{ J kg mol}^{-2}$ . For the  $\alpha$ -amino acids no such pairwise interaction parameters could be determined, as there was no linear relationship between  $\ln(k_m/k_{m=0})$  and the molality of the cosolute. If, however, the rate retardations at 1 molal were plotted *versus* the number of CH groups in the amino acid side chain, an excellent linear correlation was obtained above and below  $n(\text{CH}) = 6$ , with a larger slope above  $n(\text{CH}) = 6$ , the latter being similar to the slope obtained for the alcohols. The smaller slope at  $n(\text{CH}) < 6$  is interpreted in terms of diminished hydrophobic interactions of the side chains with the reactant due to the extensive hydration shell overlap with the hydration shells of the amino and carboxylate groups. This explanation also rationalises the results obtained for the isomers Val/*n*-Val and Ile/Leu, where *n*-Val and Leu have chain elongation and chain branching, respectively, which is outside the ionic hydration spheres and which induces a larger rate-retarding effect.

The  $\alpha$ -amino acids with an aromatic side chain do not fit into the additivity scheme. Previously we have shown that these cosolutes can give rise to pronounced kinetic medium effects due to their substantial hydrophobic nature.<sup>15</sup>

Results obtained for *N*-methylated glycines indicate that the destructive effect of ionic hydration on hydrophobic hydration is more pronounced than that of non-ionic polar hydration.

Data on isomeric dipeptides showed once more the importance of ionic hydration on hydrophobic hydration of nearby apolar groups.

The present results provide clear insight into the hydration of  $\alpha$ -amino acids and their interactions with other solutes in aqueous solution, which is a prerequisite for an ultimate understanding of complicated processes like protein-folding and enzyme-substrate recognition.

### Acknowledgements

Silvia Portolan has contributed to this investigation within the framework of the Erasmus exchange program by providing the kinetic data for the alcohols. The research was supported by the Netherlands Foundation of Chemical Research (SON) with financial aid from the Netherlands Foundation for Scientific Research (NWO).

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